

13 Search for population-specific CFTR mutations: How to avoid the identification of unclassified variantsJ.P. Altieri¹, C. Templin¹, C. Guittard¹, M. Claustres¹, M. des Georges¹.¹Laboratoire de Génétique Moléculaire, CHU, Montpellier, France

More than 1600 different sequence variations have been reported in the CFTR gene and the mutational spectrum varies in accordance with geographic and/or ethnic origins of patients.

According to the recommendations from best practice guidelines, it is advisable to offer to patients/relatives/partners a mutation detection rate higher than 80% including population-specific mutations with relative frequencies above 1%. Additional mutation screening using scanning methods (DGGE, DHPLC, HRM) or sequencing should be performed to complete the search of the most frequent mutations by commercial kits, however they permitted the identification of rare/unclassified variants. As the results of a genetic test can have serious implications for an individual and his family, it is important to identify, particularly in carrier screening or in case of suspicion of CF in fetuses with bowel hyperechogenic, only CF-causing mutations.

We designed 3 population-specific (North African, Spanish and Italian) home-made kits based on SNaPshot method (single nucleotide primer extension with dideoxy nucleotide, migration on 3130 Genetic Analyser and interpretation of results using GeneMapper Software from Applied Biosystem). According to literature data, each panel includes mutation subsets (6 to 9 different disease-causing mutations) shown to be frequent in French subjects with foreign origins and allows the identification of both wild type and mutant alleles.

In order to avoid misinterpretation of unclassified missenses or putative splice mutations, only variants of proven or certain clinical relevance are included in these panels. This accurate, specific, sensible and rapid method can easily be adapted and developed to other population-specific mutations.

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15 In vivo and in vitro effect of E831X mutationA. Hinzpeter¹, C. Costa³, Y. Alembik⁴, L. Weiss⁴, M. Goossens^{1,3}, E. Girodon³, P. Fanen^{1,2}. ¹INSERM U955, Equipe 11, Creteil, France; ²Université Paris 12, Faculté de Médecine, Creteil, France; ³AP-HP; CHU H. Mondor, Génétique, Creteil, France; ⁴CRCM, Strasbourg, France

Nonsense mutations are usually linked with severe disease; however atypical mild pulmonary illness with severe pancreatic insufficiency has already been described in CF patients with two nonsense mutations. Very mild CF was diagnosed in two siblings homozygous for the mutation E831X, located at the first nucleotide of exon 14a. Now aged of 29 y (woman) and 13 y (boy), they are pancreatic sufficient, have very mild pulmonary manifestations but have positive sweat tests. Screening of the 27 exons failed to identify another mutation.

RT-PCR performed on mRNA obtained from nasal brushing has shown the presence of one full-length and one exon 14a deleted mRNA isoforms as confirmed by direct sequencing. The first isoform would lead to a half-CFTR (after the R domain) and the second one would result in an in-frame exon 14a skipping. This latter form is predicted to produce a short deletion (from aa 831 to 872) of a cytoplasmic portion between the R domain and the seventh transmembrane segment. Two stable cell lines expressing each mutated protein have been established and biochemical analysis performed. Immunoblot revealed a truncated protein at the expected size for E831X-CFTR while CFTR del(831–873) generated a core-glycosylated protein. We have performed a blue native (BN)/SDS-PAGE showing a lower mobility (i.e. high molecular weight) of the truncated protein suggesting its possible multimerization. This could account for the very mild phenotype observed, however functional studies of both CFTR isoforms are ongoing to further characterize this mutant.

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14 Comprehensive analysis of the French NBS cohort: Excellent mutation detection rate despite high allelic heterogeneityM. des Georges^{2,1}, E. Girodon³, M.P. Audrézet⁴, T. Bienvenu⁵, E. Bieth⁶, D. Cheillan⁷, A. Iron⁸, A. Kitzis⁹, G. Lalau¹⁰, M.C. Malinge¹¹, E. Houssin¹, M. Roussey¹, A. Munck¹. ¹AFDPHE, Paris, France; ²CHU, Montpellier, France; ³CHU, Créteil, France; ⁴CHU, Brest, France; ⁵CHU, Cochin, France; ⁶CHU, Toulouse, France; ⁷CHU, Lyon, France; ⁸CHU, Bordeaux, France; ⁹CHU, Poitiers, France; ¹⁰CHU, Lille, France; ¹¹CHU, Angers, France

NBS for CF was implemented throughout France in 2002 using a four-tiered strategy: IRT1/DNA analysis (CF30 Elucigene Kit)/IRT2/ST.

Then, complete scanning and search for large rearrangements were performed in infants presenting positive or borderline sweat tests in which both mutations had not been identified by the kit. All data were collected by the French Association for NBS (AFDPHE).

987 CF neonates were diagnosed. The CF30 kit offered an excellent detection mutation rate: 86% (over 80% for all of France except for two regions); at least one mutant allele was identified in 98% and two in 75%.

273 alleles were subjected to exhaustive genotyping analysis, enabling identification of 99.3% of mutant alleles. French allelic heterogeneity was confirmed: 190 different mutations, 260 different genotypes.

The spectrum of CFTR mutations was significantly different from that reported in CF patients diagnosed on clinical symptoms (Claustres, 2000): lower F508del percentage (62.6%), higher rates of mutation associated with milder phenotypes (R117H; 7T: 4.4%, L206W: 0.8%, R347H: 0.5%), and unclassified rare variants. TIR/DNA strategy enabling very early diagnosis of classical forms of CF was successful. However, we detected up to 14% of neonates with mild or rare unclassified variants whose individual outcome cannot be accurately predicted. Collaborative studies (clinical, epidemiological and functional) to better assess the pathogenicity of these variants are underway and will be discussed.

16 Impact of the polymorphic variant 5'FR/G-260C in the Multidrug Resistance-associate Protein-1 gene on severity of cystic fibrosisA. Mafficini², M. Ortombina¹, I. Sermet-Gaudelou⁴, P. Lebecque³, T. Leal³, A. Leonard³, G. Reyckler³, K. Dahan³, X. Papermans³, G. Lenoir⁴, C. Sorio², P. Iansa¹, B.M. Assael¹, P. Melotti¹. ¹Cystic Fibrosis Center, Azienda Ospedaliera di Verona, Verona, Italy; ²Department of Pathology, University of Verona, Verona, Italy; ³Cliniques St Luc, Université Catholique de Louvain, Brussels, Belgium; ⁴"Necker-Enfants-Malades" Hospital, Paris, France

In the ATP binding cassette (ABC) transporters superfamily, MRP (Multidrug Resistance Protein)-1 shares the closest homology with CFTR, which is defective in CF disease. Functional replacement of CFTR by MRP1 has been previously suggested. We investigated the possible association of MRP1 promoter 5'FR/G-260C polymorphism with severity of CF disease. 130 non-CF subjects and 234 CF patients homozygous for F508del mutation were genotyped by snapshot: no significantly different allelic frequencies between groups were found. Association of polymorphism with disease severity as assessed by FEV1, BMI, diabetes and *Pseudomonas aeruginosa* chronic infection was not statistically significant. The CC genotype tended to be linked to higher rate of chronic colonization by PA as well as with earlier chronic colonization by PA but these trends did not reach statistical significance. Allelic frequencies in 20 CF patients were not in relation with MRP1 mRNA levels and basal/cAMP-stimulated anion conductance in nasal epithelial cells. Gene reporter assays were performed in two CF and one isogenic non-CF airway cell lines: no relevance of the 5'FR/G-260C variant was found for the MRP1 promoter transcriptional activity.

Our results did not show any statistically significant impact of MRP1(5'FR/G-260C) polymorphism on disease severity and MRP1 transcription.

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